PHYSIOLOGICAL ASPECTS OF GENETICS[1,2]

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Gene Duplication

A considerable amount of evidence indicates that desoxyribonucleic acid is capable of duplicating itself, a property also possessed by genes. (By a self-duplicating material, we mean one which plays some essential role in its own production.) Watson & Crick (1) have proposed a new structure for desoxyribonucleic acid which not only takes into account the existing analytical and x-ray diffraction data but also seems capable of explaining the mechanism of duplication. Their model consists of two helical chains coiled around the same axis, the purine and pyrimidine bases on the inside, the phosphate groups on the outside. The chains are held together by hydrogen bonds between the bases, the adenine residues of either chain being bonded specifically to thymine in the other, and similarly guanine to cytosine. The sequence of bases along one chain is not restricted, but once fixed the sequence along the other chain is determined. This complementarity, which is the most novel feature of the structure, suggests that duplication takes place by separation of the two chains, followed by the synthesis of its complement alongside each chain. The model is supported by recent x-ray diffraction studies (2, 3).

Virus Genetics

Bacteriophage.—The papers presented at a symposium on Bacteriophage, held at Royaumont, France, in the summer of 1952 have been published (4).

The following composite picture of the life cycle of bacteriophage emerges from recent studies on coliphage T2 by Hershey & Chase (5) and Visconti & Delbrück (6). The infective particle, tadpole-like in shape, consists of a protein coat or membrane surrounding a core which contains DNA.[2] The particle attaches to the bacterial cell by the end of its tail. Hershey & Chase have established by isotopic labelling experiments that the DNA passes into the bacterium, whereas the protein membrane remains outside and apparently does not participate in subsequent events. This separation of protein and nucleic acid components probably accounts for the observation of Doermann (7) that infective phage particles cannot be recovered from infected bacteria during the first half of the latent period. Once inside, the non-membrane material, now called "vegetative phage," multiplies. If the cell has been initially infected with two or more genetically marked phages,

[1] This review covers the period January 1, 1952 to May 30, 1953.

[2] The following abbreviations are used in this chapter: DNA (desoxypentosenucleic acid); FA (filterable agent).

recombination of genetic determinants (genes) occurs. This is believed by Visconti & Delbrück to result from random pairwise "mating" of vegetative particles, an event which becomes frequent after a high density of particles has been produced by multiplication. A particle is not restricted to a single mating but may undergo several matings, or none. Soon after mating starts, a process of random sorting-out and maturation of vegetative phage particles begins. Maturation consists in the conversion of vegetative into infective phage, including the acquisition of the protein coat. Maturation is an irreversible process in the host cell, and it proceeds at a constant rate. On the average, mature particles have undergone five rounds of mating. A phage cross, on this theory, is thus a problem in population genetics in which the results of single matings cannot be studied directly. Otherwise, the picture is distinctly Mendelian, involving genes, segregation, recombination, and linkage.

The above picture has little in common with an earlier theory, reviewed in previous articles in this series, according to which the phage particle breaks up into genetic subunits which multiply independently of each other and finally reassemble to form complete phage. This hypothesis has been reexamined by Dulbecco (8) and found incapable of accounting for the phenomenon (multiplicity reactivation of ultraviolet inactivated phage) which it was originally designed to explain.

For further information on the genetics and physiology of bacteriophage the reader is referred to the symposium papers mentioned above.

Viruses of higher organisms.—Burnet *et al.* (9) have obtained genetic recombination between two pairs of alternative characteristics in influenza virus. The authors decline to speculate on the mechanism of the interaction, except to state that simple mutation is excluded.

Bacterial Genetics

Genetic mechanisms.—A new mechanism of genetic exchange has been reported in *Salmonella* by Zinder & Lederberg (10). It consists of the transfer of genetic material from cell to cell through the mediation of a particulate, filterable agent (FA). Exposure of *Salmonella* to FA from another strain induces heritable transformations ("transductions" is the term favored by the authors) in the exposed cells. A variety of characters has been transformed, including nutritional, fermentative, drug resistance, and antigenic traits. Although only a single character is transformed at a time, the possibility that FA acts as a nonspecific mutagen is ruled out by the fact that the transformations are limited to the characteristics of the cells from which the FA is derived. The most plausible hypothesis is that FA is a temperate (i.e., nonlytic) phage to which bits of genetic material from the host cell may adhere and by which they may be transferred to other cells. The *Salmonella* system thus resembles both the well-known transforming principles of *Pneumococcus* and the phages.

As a result of the work of Lederberg & Tatum, Newcombe, Rothfels, and

others, it has been generally thought that genetic recombination in *Escherichia coli* K-12 can be accounted for on the basis of regular zygote formation and meiosis, although it is necessary to assume highly irregular chromosome pairing (or, alternatively, strong negative interference) in order to make sense of the genetic data (11). This concept has now been challenged by Hayes (12, 13, 14), who has found evidence that recombination in *E. coli* involves a unidirectional transfer of genes from one parent (called F+) to the other (F−). F+ cells are distinguishable from F− cells on the following criteria: (*a*) The fertility of F+ is not affected by concentrations of streptomycin which prevent their growth, whereas F− cells lose fertility *pari passu* with reduction in viable cell count; also, the fertility of F+ is increased by doses of ultraviolet which decrease the fertility of F−. (*b*) The crosses F+×F− and F+×F+ are fertile, but F−×F− is sterile. (*c*) In crosses between F+ and F− the F− parent invariably makes the greater contribution to the genotype of the progeny.

Hayes interprets these findings to mean that F+ represents an infectious, nonlytic agent which can become associated with a part of the genetic material of the cells it inhabits and thus act as a vector in the transfer of genes from F+ to F− cells. Watson & Hayes (47) consider it likely that F+ transfers intact chromosomes, of which they have provisional evidence for three. The Hayes interpretation brings *E. coli* genetics into line with the *Salmonella* system described above. One difference is that F+ is not readily separated from the cells, so that, unlike FA,[2] its transmission requires cell-to-cell contact. Cavalli, Lederberg, & Lederberg (15) are in agreement with Hayes on the essential facts, but they prefer to consider F+ and F− as sex compatibility states, the former of which is transmissible by means of the virus-like agent, F. They reject the idea that F is a carrier of genetic material. The fact of unequal genetic contributions of the two parents they explain as resulting from chromosome elimination on the F+ side. They admit, however, that zygote formation has not been proven in *E. coli* and that existing chromosome maps may be invalid.

Other transformations.—Alexander & Leidy (16) have continued their investigations of transformation in *Hemophilus influenzae* with a report on the induction of streptomycin resistance by an extract from a resistant strain. In another study (17) they present evidence for interaction betwen transforming agents from different strains to produce a new type of cell. Zamenhof *et al.* (18) have shown that DNA is an essential constituent of the active principle.

Origin of bacterial variants.—A new approach to the problem of whether the acquisition of resistance to phage and antibiotics is spontaneous or induced has been devised by the Lederbergs (19). Their method differs from previous ones in that it makes it possible by a simple technique to isolate the mutants before they have come into contact with the agents. Induction is ruled out in these cases, in agreement with previous analyses. Ryan (20) has concluded that the adaptation of a strain of *E. coli* to grow on lactose

results from selection of spontaneous mutants. Eagle *et al.* (21) consider that the slightly enhanced resistance which develops in bacteria exposed to low concentrations of antibiotics may result from induction. Some new methods in connection with the selection of resistant mutants have been described by Bryson & Szybalski (22). They reiterate earlier findings that resistance to most antibiotics is acquired in steps, leading to the therapeutically interesting conclusion that antibiotics will usually eliminate all members of a bacterial population if used initially in high concentrations. Yudkin (23) has devised a theory called "clonal variation" to explain the acquisition of drug resistance; stated in qualitative terms, it appears to differ from existing theories, but it seems likely that further elaboration will show that it is indistinguishable from a spontaneous cytoplasmic mutation hypothesis.

Induced mutations in bacteria.—Demerec and co-workers (24, 25) have carried out further studies on the phenomenon of delayed appearance of mutations. They had previously shown that up to 12 cell divisions may be required following ultraviolet treatment of *E. coli* before the rate of mutation to phage resistance returns to its normal value. They now find a delayed appearance of induced reversions to nutritional independence following treatment of amino acid deficient mutants with ultraviolet or manganous chloride. They consider that the most plausible explanation of the effect is the induction of a gene instability which may persist through a number of cell divisions. The same authors have reported a new phenomenon, "mutagen stability," referring to the failure of mutagens to increase the rate of reversion of certain amino acid deficient mutants over the spontaneous rate. Finally, they have found considerable evidence that mutation in *E. coli* is intimately connected with cell division, in apparent contradiction with earlier results of Novick & Szilard which indicated a constant mutation rate per unit time, but in agreement with conclusions from the genetics of higher organisms. [See also Witkin (46)].

Newcombe (26, 27) is of the opinion that none of the mechanisms which have been proposed to explain the delayed appearance of ultraviolet-induced, phage-resistant mutants in *E. coli* are satisfactory and suggests that a systematic selection artifact is responsible for the phenomenon. With respect to the apparent discrepancies in the measurements of mutation rates, mentioned above, he suggests that these can be resolved by the hypothesis that mutation results from an error at the time of gene duplication, with the additional assumption that the probability of occurrence of an error is determined by the concentration of a chemical substance produced in the cell. That chemical substances may in fact be partly responsible for spontaneous mutations is supported by the finding of Novick & Szilard (28) that the spontaneous rate of mutation to phage resistance is reduced by one-half to two-thirds in *E. coli* when the medium is supplemented with guanosine.

Adelberg & Myers (29) have described a new, very efficient penicillin technique for the selection of biochemically deficient mutants of *E. coli*.

GENETICS OF *Neurospora* AND OTHER ASCOMYCETES

Genetically, *Neurospora* is much more like the higher plants and animals than it is like the bacteria. There is no evidence that transforming agents of either the *Pneumococcus* or *Salmonella* type have a role in *Neurospora*, although the synthesis and resolution of heterocaryons should provide an ideal means of detecting them. The fact that none have been found by this method suggests that they are rare or nonexistent in *Neurospora*, or else that they are formed as a result of cell breakage and exist only in lysates.

Cytoplasmic inheritance.—A case of cytoplasmic inheritance in *Neurospora* has been described by Mitchell & Mitchell (30). The character, called poky, is transmitted to all ascospores formed in poky perithecia and to few or none in nonpoky perithecia. The evidence is such as to remove any reasonable doubts as to its cytoplasmic nature. Phenotypically, poky is characterized by its slow growth rate. Biochemically, it has been found by Haskins *et al.* (31) to be deficient in cytochromes-*a* and *b* and in cytochrome oxidase and succinic oxidase activity. Young cultures contain an excess of cytochrome-*c*. Biochemically and genetically, poky resembles to a marked degree the *petite colonie* mutation in yeast, studied by Ephrussi and co-workers.

It is an interesting and possibly significant fact that the two most widespread porphyrin systems, the chlorophyll and cytochrome systems, have been found to have cytoplasmic components in their inheritance. This of course does not exclude chromosomal components also; and, in fact, chromosomal genes are known to affect both systems.

Suppressors.—Yanofsky (32) has reported a mutation which suppresses a tryptophan-requiring strain lacking the enzyme necessary for the synthesis of tryptophan from indole and serine. The suppressed mutant grows on minimal medium and produces the enzyme (about 5 per cent as much as wild type). The most interesting point is that the suppressor does not suppress another tryptophan mutant also lacking the tryptophan-synthesizing enzyme. The second tryptophan mutant behaves as an allele of the first in crosses and in heterocaryon tests. It can be concluded that the action of the suppressor may not be simply to take over the function of the normal allele of the suppressed mutant. Rather, it may be supposed, the suppressor partly restores to the mutant allele, or to its product, the ability to function, presumably by altering some critical factor in the intracellular environment [Horowitz (33)]. Since the extent to which the system can be reactivated will depend on the amount and kind of damage which it has suffered, it is easily understood, on this theory, why a suppressor may affect only certain alleles at a locus. This interpretation is supported in the particular case under discussion by the fact that the suppressor of tryptophanless has a deleterious effect on the growth of wild type.

Mitchell & Mitchell (34) have tested a suppressor of pyrimidineless against a number of mutant genes of the proline-ornithine and lysine series in *Neurospora*. They find that the suppressor can interact with the mutants

in various ways, sometimes acting as a suppressor, sometimes as an enhancer of the phenotype.

It is not necessary to assume that all suppressors act in the same way. Lein & Lein (35) have evidence that a suppressor of three nonallelic acetate-requiring mutants acts by opening a secondary pathway of acetate synthesis.

"Grigg effect."—Grigg (36) has found that growth of conidia of wild type *Neurospora* on sorbose-minimal medium is inhibited if the plates are heavily seeded with mutant conidia. He pointed out that this could invalidate the much used back-mutation method for assaying mutagenic activity. Grigg's conclusion was disputed by Kölmark & Westergaard (37), who pointed out that the conditions of Grigg's experiments (the use of a sorbose medium to produce colonial growth) were not the same as those of the back-mutation test (standard medium, with a genetically colonial strain). Under the latter conditions, it was reported, no "Grigg effect" was observed. Stevens & Mylroie (38), however, in a fairly extensive series of trials, obtained the effect under the standard conditions of the back-mutation test, but not with all mutants. Their results suggest that an essential condition of the phenomenon is that the mutant conidia be capable of surviving on minimal plates for prolonged periods; conidia from some strains die out rapidly and do not give the effect. Stevens & Mylroie failed to confirm Grigg's claim that the back-mutations picked up in the standard test are entirely of spontaneous origin. Jinks (39) has suggested that the "Grigg effect" is responsible for the disturbed linkage relationships observed in *E. coli*, an idea which Ryan (40) disputes on technical grounds. In any case, the discrepancies referred to by Jinks seem much better accounted for on the basis of the F-mechanism described earlier.

Pseudo-wild types.—Mitchell *et al.* (41) have found that the phenotypically wild progeny derived from crosses between closely linked mutants in *Neurospora* are frequently not genotypic wild types, as shown by the fact that both original mutants segregate out in crosses to true wild types. Considerable evidence indicates that these pseudo-wild types originate as disomics formed by occasional nondisjunction during meiosis. The disomic condition is apparently unstable in mitosis, so that pseudo-wild type cultures are heterocaryotic, containing haploid and possibly some disomic nuclei. This discovery is obviously important in connection with crossover or pseudoallele studies in *Neurospora*.

Other Ascomycetes.—Following on the successful production of diploid *Aspergillus* by Roper (42), Pontecorvo *et al.* (43) have managed to carry out a genetic analysis of the asexual species, *Aspergillus niger*. They take advantage of the fact that the diploidized strains undergo somatic crossing over. A review of *Aspergillus* genetics, containing many previously unpublished data, has appeared (85).

Lindegren (44) has presented a statement of his views on the nature of the gene. Roman & Sands (45) show that diploidization occurs spontaneously in haploid clones of *Saccharomyces* and that the diploid cells can give

rise to triploid and tetraploid zygotes. These could easily yield the aberrant ascospore ratios on which Lindegren bases his system.

Genes and Enzymes

Examples of mutations which can be shown by direct analysis to lead to a deficiency of specific enzymes have multiplied in the period under review. In addition, two cases have been reported in which a genetic change has produced a qualitative modification of enzyme structure. In following this work it is well to bear in mind that the term "mutant" is an ambiguous term covering any inherited change, whether of nuclear or cytoplasmic origin and whether genic or chromosomal. In bacteria, despite recent advances, it is not known to what extent these distinctions are valid, and in most of the biochemically important bacterial mutants no genetic analysis has been done or is feasible. On the biochemical side, it should be pointed out that what appears to be an enzyme deficiency may actually be an enzyme inhibition, or even an artifact resulting from destruction of the substrate by some competing system. These sources of error can be, but are not always, controlled.

E. coli.—Maas & Davis (48) have investigated the enzymatic synthesis of pantothenic acid in extracts of wild type and two mutant strains of *E.coli*. Whereas the wild type extract catalyses a rapid synthesis of the vitamin from β-alanine and pantoic acid (49), one of the mutants, characterized by an absolute requirement for pantothenate, shows no enzyme activity either in extracts or whole cells. The other mutant, characterized by a pantothenate requirement only at temperatures above 30°C., produces a highly thermolabile form of the enzyme. No evidence of interaction was found when wild type and mutant extracts were mixed, indicating that the difference in stability is attributable to structural differences between the enzymes, rather than to differences in the amounts of destructive or protective agents in the extracts.

Other cases in *E. coli* include an ornithineless mutant which is deficient in an enzyme for converting N-γ-acetyl-L-ornithine to L-ornithine, studied by Vogel (50); three lysineless mutants reported by Dewey & Work (51) to lack detectable diaminopimelic acid decarboxylase activity; four strains unable to ferment lactose, reported by Lester (52) to produce significantly less β-galactosidase activity than the wild type; and a mutant with a requirement for isoleucine and valine, shown by Rudman & Meister (53) to be deficient in the principal transaminase system for these two amino acids. In every case there is reason to believe that the enzymatic deficiency is the cause of the nutritional defect. The findings of Rudman & Meister are of special interest, since the isoleucine-valine mutants have for some time been regarded as having a primary block only in the synthesis of isoleucine; the valine requirement was imagined to result from an inhibition of valine synthesis by the accumulating precursors of isoleucine. Much circumstantial evidence seemed to support this view [see, for example, Umbarger & Magasanik (54)]. This concept now appears unnecessary, at least insofar as it has been applied

to the transamination step in the two syntheses. This case provides a good example of the hazards involved in basing conclusions on indirect evidence.

Leupold & Horowitz (55) have published the details of an analysis of 161 temperature mutations of *E. coli*, leading to the conclusion that the method used for detecting biochemical mutants does not selectively favor the recovery of unifunctional types to a sufficient degree to account for the high frequency of such types actually found. The results thus fail to support the view that the one gene-one enzyme relationship is an artifact based on a selected group of mutants.

Neurospora.—Horowitz & Fling (56) have found a pair of alleles in *Neurospora* which govern the thermostability of the enzyme tyrosinase. One of the alleles determines a thermostable form of the enzyme, the other a thermolabile form. It was concluded that a structural dissimilarity exists between the enzymes on the basis of three kinds of evidence: purification studies, a kinetic analysis of the thermal inactivation reaction, and experiments with mixtures of the two enzymes. The authors point out that no conclusions can be drawn as to the mechanism of enzyme synthesis from these results, since they are consistent both with the template theory and also with the theory which assumes that one form of tyrosinase is a precursor of the other. In a study of the properties and occurrence of *Neurospora* tyrosinase, Horowitz & Shen (57) found that the synthesis of tyrosinase is not confined to one mating type, contrary to the results of some previous workers.

Evans & Nason (58) have described a nitrate reductase system in *Neurospora;* the enzyme (or enzymes) is lacking in certain mutants unable to utilize nitrate as a source of nitrogen. Only a preliminary report is available at the present writing.

Yeast.—Ycas & Starr (59) have described a mutant of *Saccharomyces cerevisiae* lacking cytochromes-*a*, *b*, and *c*, and containing only traces of catalase when grown on minimal medium. Addition of glycine or protoporphyrin IX results in formation of cytochrome-*c* and catalase, but not cytochromes-*a* and *b*. Genetic analysis showed that the glycine requirement is inherited as a single gene difference, whereas the cytochrome-*a* and *b* deficiency is inherited as a cytoplasmic factor. It is concluded that the strain carries a partial genetic block in glycine synthesis, resulting in a deficit of cytochrome-*c* and catalase; and, in addition, lacks the cytoplasmic factor for cytochrome-*a* and *b* production.

More inferential in nature are conclusions drawn from experiments in which the rate of some vital activity is taken as a measure of enzyme activity. In yeast, the fermentation of carbohydrates by living cells is conveniently assayed, and a number of papers have appeared in which genetic aspects of fermentation have been studied. Several reports from Lindegren's laboratory (60, 61, 62) have dealt with the genetic control of oligosaccharide fermentations. The enzymes being investigated are presumed to be carbohydrases of various kinds. Similarly, Spiegelman & DeLorenzo (63) have studied the inheritance of the ability to ferment galactose. They conclude

that the ability to initiate production of the galactose fermentation system ("galactozymase") is inherited in a Mendelian way, but that once established in the cell the system is self-perpetuating in the presence of galactose. It is not known what component, or components, of "galactozymase" is actually involved here.

Mammals.—Phenylpyruvic oligophrenia, an hereditary form of feeblemindedness in man, is characterized by the excretion of phenylpyruvic acid in the urine (phenylketonuria). Jervis (64) has carried out experiments with livers from normal and affected individuals and reports that he was unable to detect, in the affected livers, the enzyme system which converts phenylalanine to tyrosine. He concludes, perhaps prematurely, that absence of this system is the essential metabolic lesion of the disease. The fundamental work on the system was done by Undenfriend & Cooper (65). It is evidently complex, involving at least two enzymes. The mechanism of the reaction is unknown.

Foster (66) has compared the tyrosinase activity of foetal guinea pig skins of different color genotypes. He finds no simple parallelism between enzyme activity and the amount or kind of natural pigment. Some pigmented skins showed no detectable activity, and others with extremely reduced pigmentation showed relatively high levels of activity.

Metabolic Pathways

The application of genetics to problems of metabolism being somewhat peripheral to the main topics of this review, we shall mention only briefly some of the interesting contributions in this field.

Neurospora.—Bonner *et al.* (67) have obtained evidence that a number of mutants of the tryptophan nicotinic acid group are not completely devoid of the capacity to carry out the synthesis; 7 out of 9 strains tested showed evidence of incomplete blocking. Doudney & Wagner (68), on the basis of a study of a threonine-sensitive mutant, have arrived at the interesting conclusion that threonine and homocysteine may be precursors of the thiazole moiety of thiamine. Haas *et al.* (69) and Ames *et al.* (70) have analysed the pathway of histidine synthesis by use of a series of histidine-requiring mutants. The usual procedure of isolating accumulated metabolic products was followed, but none of the products (a series of imidazole derivatives) were active as biological precursors of histidine. The hypothesis was advanced, supported by some chemical evidence, that the actual intermediates are phosphate esters of the isolated compounds. Harris (71) has studied the interaction of thiamine and pyridoxin in growing mycelium and concludes that pyridoxin interferes with thiamine synthesis, whereas thiamine inhibits the destruction of pyridoxin. Harrold & Fling (72) have described mutants which require formate or formaldehyde for growth; adenine supports limited growth. Sheng & Sheng (73) have reported a study of carotenoid synthesis in a series of color mutants. Strauss (74, 75) has investigated various aspects of the metabolism of a number of acetate-requiring strains.

They accumulate acetylmethylcarbinol, leading to the conclusion that these mutants are unable to complete the oxidation of pyruvic acid.

Glomerella.—Markert (76, 77) has described the production of nutritional mutants in the Ascomycete, *Glomerella*. They have the convenient property of being visibly distinguishable from the parent type, making the task of selection relatively easy. Two mutants have been found which grow when supplied with glutathione, but not when given a mixture of the constituent amino acids. They grow on a mixture of cysteinylglycine and glutamylcysteine. Both mutants appear to carry chromosomal aberrations.

E. coli.—Davis (78, 79) has continued his important series on the biosynthesis of aromatic compounds. He deduces a branched pathway in which a derivative of shikimic acid gives rise in separate reactions to the following compounds: *p*-aminobenzoic acid, anthranilic acid, tyrosine, phenylalanine, and *p*-hydroxybenzoic acid. He points out that the results of the mutant study contradict in several points the conclusions arrived at by inhibition analysis of the same pathway. [For a contrasting view, see Bergmann *et al.* (80).] Utilizing another series of bacterial mutants, Vogel & Davis (81) have analysed the pathway of proline synthesis from glutamic acid. Maas & Vogel (82) have arrived at the interesting deduction that α-ketoisovaleric acid is a precursor of the pantoic acid moiety of pantothenic acid; this means that pantothenic acid and valine have a common precursor. In the course of their investigations of the structure and function of lipoic acid derivatives, Reed & DeBusk (83) produced a mutant of *E. coli* with a requirement for acetate, citrate, and succinate. These were replaceable by a conjugate of lipoic acid, identified as lipothiamide. The mutant is unable to conjugate α-lipoic acid with thiamine. Stone & Hoberman (84) have analyzed the mode of utilization of proline peptides by a proline-requiring strain. Under aerobic conditions, peptides are utilized better than free amino acids, but under anaerobic conditions peptides and free amino acids support equal growth. The authors present evidence which indicates that the effect is a result of the aerobic deamination of free proline. They conclude that peptides are not utilized in a unique manner by the mutant, but must first be hydrolysed.

Cellular Antigens

Transfusion reactions and erythroblastosis fetalis have called widespread attention to the diversity of human cellular antigens, and serological research has provided increasingly sensitive techniques for the detection and study of these inherent human differences. The reviewers are faced with an embarrassment of riches in the recent contributions to this field, and only a few categories of interest can be sampled here. Perhaps the predominant conception to be gained from this consideration is the very great complexity, and the high degree of individuality, of the antigenic characteristics of cells and of the antisera by which they are recognized. This complexity has at least three aspects: numerous genetic loci contribute to the antigenic characters of cells; a considerable extent of allelic diversity has already been recognized

at several of these loci; and the antisera which define the cellular antigens are probably seldom, if ever, as sharply specific as one might wish them to be. Studies of chemically known haptens (86) have established that a multiplicity of antibodies, varying in kind and degree of specificity, are produced in response to a single unit of antigenic structure. This antibody population can be fractionated by reaction with antigens related to the one that induced their formation, but there is no necessary simple correspondence between an antibody fraction so isolated and any particular detail of structure in the antigens. The cellular antigens controlled by a series of alleles frequently display serological interrelationships. A representation of these relationships, based on a supposition that a mosaic of sharply distinct structural characteristics shared by or distinguishing the related antigens is revealed by corresponding antibody fractions of discrete and absolute specificity, is suspect because of the patently unrealistic nature of the serological assumptions upon which it is based.

The "C-D-E" conception of the Rh complex, which involves the kind of assumption mentioned above, seems especially dubious in the light of recent developments. Recognition that the three "pairs of alternatives" postulated by this conception do not in fact encompass the variations in this series of related antigens antedates the period covered by this review. Besides the necessity of substituting such series as "C-C^{U}-C^{W}-c^{v}-c" for the simple alternatives C and c [see (87) for an earlier review], and other similar extensions, the retention of this conception has required the postulation of a chromosomal deletion to account for the failure of certain Rh antigens to give evidence of any property that might be assigned to the hypothetical "C or E loci" (88, 89), and the postulation (90) of still another "component," f, which has to date been noted only among antigens sharing the specificities that had been designated c and e. Meanwhile, Wiener *et al.* (91) have discovered an additional antigen which, if it were to be described by the C-D-E-F system, would require a deletion only at the "E locus." Rhesus monkey blood shows no test evidence of specific reaction with antibody fractions designated anti-C, -c, -D, or -E, but guinea pigs injected with monkey cells produce a good "anti-D" (92; see also 93). The assumptions that "D" must be present in order to induce antibodies subject to this designation, and that in monkey blood "D" is so situated in the cell that it cannot react with the antibodies, seem unwarranted because human Rh-negative cells, which by definition lack "D," nevertheless liberate on heating a substance that will also induce "anti-D" in guinea pigs (94). Ponder & Ponder (95) have suggested that the active antigen is a modified product of the fragmentation of the heated cells. Whether the material is native or is a product of fragmentation, it cannot on present evidence by identified with the hypothetical "substance D" but can only be described as a material that, on injection into guinea pigs, induces a pattern of cross reactive antibodies, including many similar in specificity to those found in anti-Rh_0 serum. When this heterogeneous population is fractionated by minimal absorption with Rh-negative cells, a

reagent comparable to an anti-Rh_0 reagent is obtained. These observations are entirely compatible with an interpretation of the Rh complex as a series of single, related antigens. They offer serious difficulties to an interpretation on the "CDEF" basis. In a paper also reporting other evidences of the complexity of isoimmunization and Rh typing, Wiener & Brancato (96) have described a variant of Rh_0 of unusually low avidity, which permitted the baby possessing it to display only a mild degree of erythroblastosis in spite of a high maternal antibody titer.

Coincident with the extension of the series of distinguishable Rh antigens has come a recognition of the complexity of the antisera that provide the tools for typing bloods. Cann *et al.* (97) fractionated three Rh antisera by electrophoresis convection and found Rh-reactive antibody throughout the serum globulins, including α globulin. They noted marked individual variations even in sera of the same type. In addition to differences in the variety of antigens with which they will react, antibodies can be classified in terms of their effects on positive cells in particular test systems, for example, those which produce agglutination in saline diluents, or block the saline agglutinins, or cause agglutination in the presence of antiserum to human globulin, or agglutinate trypsin or other enzyme-treated cells, or agglutinate only in colloid diluting fluids, or block the antibodies that agglutinate in such systems. It has been pointed out (98) that when mixtures of these diverse antibody types are present in an antiserum, conclusions based on tests with the serum will often depend on the particular testing technique used. The competent use of a battery of these newer techniques seems likely to reduce the incidence of transfusion reactions (99, 100).

Many of the antisera that have been studied in connection with the recognition of new antigenic variants have shown complicated mixtures of antibodies. For example, serum from the original individual displaying the postulated "deletion" (-D-) contained antibody fractions described as "anti-C, anti-c, and anti-e"; and serum from another individual of the same type (89) would react with cells having the antigenic specificities labelled e and perhaps also C, c, and E. A new Rh antibody fraction called f (90) was found in the serum of a hemophilic male who had received 35 blood transfusions; his serum also contained anti-B, -K, -S, and -N. Three of the five people in whom anti-Duffy (anti-Fy^a) antibodies have been reported were also hemophilic (101), The new blood group antibody recognizing Jk^b (an allele of Kidd) was found in a serum also containing anti-Fy^a, after transfusion and miscarriage. Clear and consistent demonstration of the anti-Jk^b activity of the serum requires the use of the indirect anti-globulin test on trypsin-treated cells (102). In a study of quantitative aspects of human antigen Fy^a, Race *et al.* (103) observed that trypsin-treated positive cells reacted to one serum only when homozygous, and that another antiserum detected a common dosage effect at this locus. There appeared to be some relationship between this antigen and Rh; on statistical grounds, Rh-negative people were too often Duffy-positive, and too often homozygous for the Duffy antigen.

Limitation of space forbids the discussion of numerous other studies related to the human erythrocyte antigens. Categories of investigation that have produced results of particular interest to the reviewers include additional studies of the nature and effects of Rh antibodies and of their transmission across the placenta (104 to 107); erythroblastosis-like phenomena in animals (108, 109, 110); population studies of Rh and other blood types (111 to 114); genetic and population studies of associations among various blood-cell characteristics and other human attributes (115 to 120); reports dealing with newly discovered erythrocyte antigens (121, 122, 123), and the nature of those already known (124, 125); the relationships among the Lewis antigens, the secretor characteristic, and the A-B-O blood groups (126, 127, 128); and studies of the normal blood groups themselves, with particular reference to the nature, origin, and interrelationships of the normal antibodies and the corresponding antigens in human blood (129 to 136) and of other, diverse origins, including other animal materials (137 to 141) and plant extracts (142, 143, 144).

Immunogenetic analyses of the erythrocytes of species other than man have continued to reveal extended multiple allelic series controlling antigens interrelated in ways that suggest complex patterns of cross-reaction. In the "B system" of cattle at least 80 different "blood groups" have been reported (145), each apparently controlled by an allele at the *B* locus. The "C system" includes at least 22 groups and alleles. Two other systems, at present much less complicated, have also been described (146), and there are in addition a number of apparently independent loci affecting cellular antigens in cattle. A similar situation prevails in chickens; the earlier work of Briles *et al.* (147) has been extended to a detailed study of the *C* and *D* blood group loci, both of which are complex (148). A point of considerable interest is the report by Shultz & Briles (149) that "natural" selection under flock conditions favored heterozygosity at the *B* locus, while "artificial" selection for high egg production favored heterozygotes at the *A* locus and probably at the *B* locus as well. It seems likely that at least part of the explanation for the great diversity of cellular antigens segregating in animal populations may rest on a physiological advantage conferred by heterozygosity for chromosomal regions marked by the antigen-controlling genes. Such selective conditions oppose the usual tendencies toward fixation or loss of alleles, and genetic heterogeneity is maintained. Restrictions of space prevent the citation of several other genetic studies relevant to this concept. With specific reference to immunogenetic heterozygosity, however, the recent work of Bryan & Miller (150) should be mentioned. Antisera to pigeon bloods heterozygous for a pair of contrasting characters (C′ and C, found in *Columba guinea* and *Columba livia* respectively) contain an antibody fraction that will react only with the cells of heterozygotes. This provides direct serological evidence of interaction producing an effect of heterozygosity per se, but whether this specificity is more properly ascribed to a substance unique in the heterozygote, or to some other more subtle consequence of the nature of antibody responses and reactions, and whether the effects observed are those

of the alleles designated or of associated chromosomal regions, remain to be established. Other pairs of contrasting characters (150, 151) in the same hybrids have given no evidences of similar allelic interactions. Irwin (152) has reviewed and extended his classic studies of evolutionary patterns in pigeon and dove antigens.

Fox & White (153) have repeated and extended earlier observations suggesting that genes at different loci may interact in affecting the specificity of antigenic materials found in *Drosophila* extracts. The important work on antigens in paramecia has included a report by Beale (154) of three loci affecting antigen variation in *Paramecium aurelia*, var. 1. Four alleles have been recognized at the *g* locus, three at *d*, and two at *s*. Antigens controlled by alleles usually display a serological relationship. Only one of the three loci is normally expressed in an individual at any particular time; which one is detectable depends on the cytoplasmic state of the cell, and the stability of these alternative states varies widely. Transformations occur when the temperature of culture is changed; these often show a prolonged delay, but the transformations when they occur are sudden.

Numerous papers in the field of bacterial, bacteriophage and virus serology must be neglected here.

Transplantation Specificity and Related Phenomena

The establishment of permanent natural transplants of hematopoietic tissue exchanged between twin bovine embryos (155, 156) has been subjected to further interesting study by Stormont *et al.*, working with cattle (157) and sheep (158). In both, the exchange is associated with freemartinism when the embryos differ in sex, but in a recent report by Dunsford *et al.* (159) of a human female erythrocyte chimaera evidently illustrating the same phenomenon, whose twin had been a male, no sexual abnormality existed. The report offers several other points of interest: for example, the male twin, who had died 25 years ago at the age of three months, could still be blood-typed because descendants of his cells comprised almost 40 per cent of his sister's erythrocyte population. Although the chimaera had in her circulation large numbers of A cells derived from her twin, her behavior as a secretor was consistent with her own tissue-genotype, and she secreted O (H) substance in her saliva, not A. Contrasting with this tissue-specific aspect of the physiology of secretion, the presence of normal antibodies in her serum was affected by the nature of the circulating cells; the chimaera was genotypically of blood group O, but no anti-A was present in her serum. The authors minimize the possibility that anti-A was being formed but constantly being absorbed by descendants of the transplanted A cells, on the grounds that the circulating A cells gave no evidence of sensitization in delicate test systems. However, the work of Anderson *et al.* (160) and Billingham *et al.* (161) on skin transplants in twin cattle suggests that cells or materials other than erythrocyte primordia are also involved in the twin embryo exchange; if so, it is conceivable that anti-A formed by the human

chimaera might be reduced below detectable levels by other tissues. Alternatively, the histocompatibility work of Snell *et al.* (162, 163) supports earlier suggestions that the presence of large amounts of antigen "paralyzes" the antibody-producing mechanism in some little-understood fashion; on these grounds the absence of anti-A in the chimaera might well represent a more fundamental effect than one of simple absorption. The autonomous nature of the A and O properties of the chimaera's erythrocytes suggests that this antigenic alternative is inherent in the blood cells and is not a secondary characteristic derived from other tissues, as was found by Stormont (164) to be true of a cattle antigen recognized by means of normal isoantibodies. The papers by Snell *et al.*, cited above, include an admirable study of the genetic control of transplant specificity in mice, involving, as with erythrocyte antigens, complex multiple allelic series with evidences of cross-relationship among the active products of alleles.

Ripley & Owen (165) have produced persistent erythrocyte mosaicism by injecting embryonic rat cells into rat embryos of different blood type. Andres (166) has injected dissociated cells from particular chick embryos into the circulation of other embryos and reports that teratomas of various composition are formed by the donor cells in the host embryonic membranes. These and earlier observations by Weiss & Andres (167) suggest that donor cells also participate in a type-specific localization within the embryonic host and are incorporated into its normal differentiation. Evidence on this latter important point appears at present to be restricted to pigment cells; the parallelism with the natural twin chimaeras and with the experimentally produced rat mosaics is, however, striking. Chiakulas (168) reports that in amphibia intimate host-graft fusion occurs only between tissues normally associated in an intact organism; such tissue-specific cell affinities tend to support the conception of organization described by Weiss as "molecular ecology." Prehn (169), however, reports that tumor transplants into skin grafts indicate no intrinsic incompatibility between fixed tissue of different genetic origin; these incompatibility reactions appear to be systemic rather than local interactions between dissimilar, adjacent tissues. Smith *et al.* (170) report that virus-induced rabbit papillomas grow well when transplanted to suckling young, in contrast with their uniform failure when transplanted to adults. Space is too limited here to permit reference to numerous other studies of tumor transplantation.

Other Genetic Characteristics of Physiological Interest

Hemoglobins.—Great interest attaches to the recognition that human hemoglobin is subject to straightforward genetic modification. Several recent reviews [e.g., (171 to 174)] have dealt with this material and permit presentation of a general picture here without separate citation or discussion of the numerous original papers. Two systems of hemoglobin synthesis are recognized, one leading to the formation of fetal hemoglobin (F) and the other, replacing the first during normal development, leading to normal adult

hemoglobin (A). F and A are sharply distinct, particularly in terms of their serological specificities (175, 176) and their susceptibility to alkaline denaturation. Either a genetic block to the synthesis of A (exemplified by the allele for thalassemia) or environmentally induced anemia apparently leads to the maintenance or reactivation of the fetal mechanism, so that the hemoglobin of individuals homozygous for thalassemia is indistinguishable from F. Anemic individuals often have some F in addition to A, and serological evidence has suggested that even individuals classified as normal adults may have small amounts of F (175). The adult mechanism is subject to several genetic deviations; the most frequent of these is effected by the sickle-cell allele, responsible for the formation of an electrophoretically distinguishable hemoglobin (B). The cells of heterozygotes sickle and contain both kinds of hemoglobin, the quantity of A predominating over that of B. Homozygotes are sickle-cell-anemics and have B but no A; they usually also have varying amounts of F presumably resulting from a compensatory reactivation of the fetal mechanism like that found in other types of anemia. The relative amounts of B and A in sickle-cell heterozygotes vary in different individuals; this quantitative variation appears to have a strong genetic component, but its basis is at present uncertain. Itano (177) has presented evidence that "isoalleles" of the normal alternative may differ in their efficiency in competition with the allele governing hemoglobin B synthesis. The allele for thalassemia is clearly not at the sickling locus; it nevertheless evidently provides a more complete block to the synthesis of A than of B, since doubly-heterozygous individuals (for sickling and thalassemia) show a predominance of B over A, together with varying amounts of F (178). An explanation consistent with these data would place the block effected by the thalassemia allele after the point at which the sickling locus acts. It seems possible that a sequence of gene-affected reactions in the synthesis of hemoglobin may emerge from future work along this line. Meanwhile, additional abnormal adult hemoglobins (C, D) have been shown to be simply inherited. The genetic relationships among the aberrant adult hemoglobins have not been definable by the data at hand; more general biochemical-genetic considerations might suggest the hazardous guess that an extended multiple-allelic series, including several alleles giving qualitatively aberrant products as well as those resulting in essentially quantitative variations (the normal isoalleles), is involved. The genic action in the synthesis of aberrant hemoglobins seems clearly to affect the synthesis of the globin, not of the heme (179, 180). Population studies of the sickling characteristic offer some challenging problems (181 to 185). Somewhat related papers of genetic interest dealing with hemoglobin in sheep (186) and in mice (187) might well be singled out for specific mention, from among the numerous papers at hand.

Hemophilia.—The general term "hemophilia" has been shown to include several different disorders, primarily by the application of a technique familiar in biochemical genetics. Plasma or serum from one hemophilic individual may restore the clotting property to plasma of another; it follows that different components of the clotting system are deficient in the two individuals.

At least three plasma thromboplastin factors account for at least three types of hemorrhagic disease (188): absence of AHG (anti-hemophilic globulin) is corrected by barium sulfate treated normal plasma but not by serum; absence of PTC (plasma thromboplastin component) is corrected by normal serum but not by barium sulfate treated normal plasma; and PTA (plasma thromboplastin antecedent) deficiency is corrected by either of the materials mentioned. Several papers (189 to 194) have dealt with these and perhaps other hemophilia-like disorders and their bases. Genetic data are at present insufficient to ascertain how these deficiencies are related in inheritance or, in fact, whether some of them are inherited at all. Methods currently at hand do not appear to be sufficiently sensitive to detect the relatively slight clotting defect which may be characteristic of female carriers of traditional hemophilia (195). There has, however, been reported a case of true hemophilia in a woman (196). Circulating anticoagulants that appear after repeated transfusion of hemophilic persons have been subjected to further study (197, 198); the specificity of this apparently immune response may in the future provide additional tools for the identification and study of genetic variations in this class of disorders.

Other physiological traits.—An apparent homozygote for the Pelger-Hüet anomaly of leucocytes has been described (199, 200). Although the nuclei of granulocytes were like those in rabbits displaying a very similar anomaly, the condition is evidently not lethal when homozygous in man, as its prototype is in rabbits. Hereditary spherocytosis has been shown to depend upon a Mendelian dominant; the characteristic shows a higher incidence in men than in women and appears to be associated with leg ulcers and gallstones (201). The inheritance of oval erythrocytes, and possible linkage with other blood factors, has been reported by Bird & Bailey (202). An extensive study of the genetics of diabetes mellitus (203) has led to the conclusion that a simple autosomal recessive is responsible for this disorder. The frequency of the recessive allele is estimated to be between 0.2 and 0.25. A simple recessive, fully penetrant, is believed to be responsible for fibrosis of the pancreas (204). The disease is rather frequent (0.7 to 1.0 per 1000 live births), and either a high mutation rate or some degree of superiority of the heterozygote is postulated as a basis for this high frequency. The reader is referred to the excellent bibliographies published in each issue of the *American Journal of Human Genetics* for further citations of human genetic studies of physiological interest.

We must neglect many interesting reports of genetic studies in other animals and in plants, but reference should be made to the publication of the second edition of Grüneberg's *Genetics of the Mouse* (205). The book presents and explores numerous important physiological and developmental facets of its subject. Heston (206) has reviewed the relations of mouse genetics to our understanding of human cancer.

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